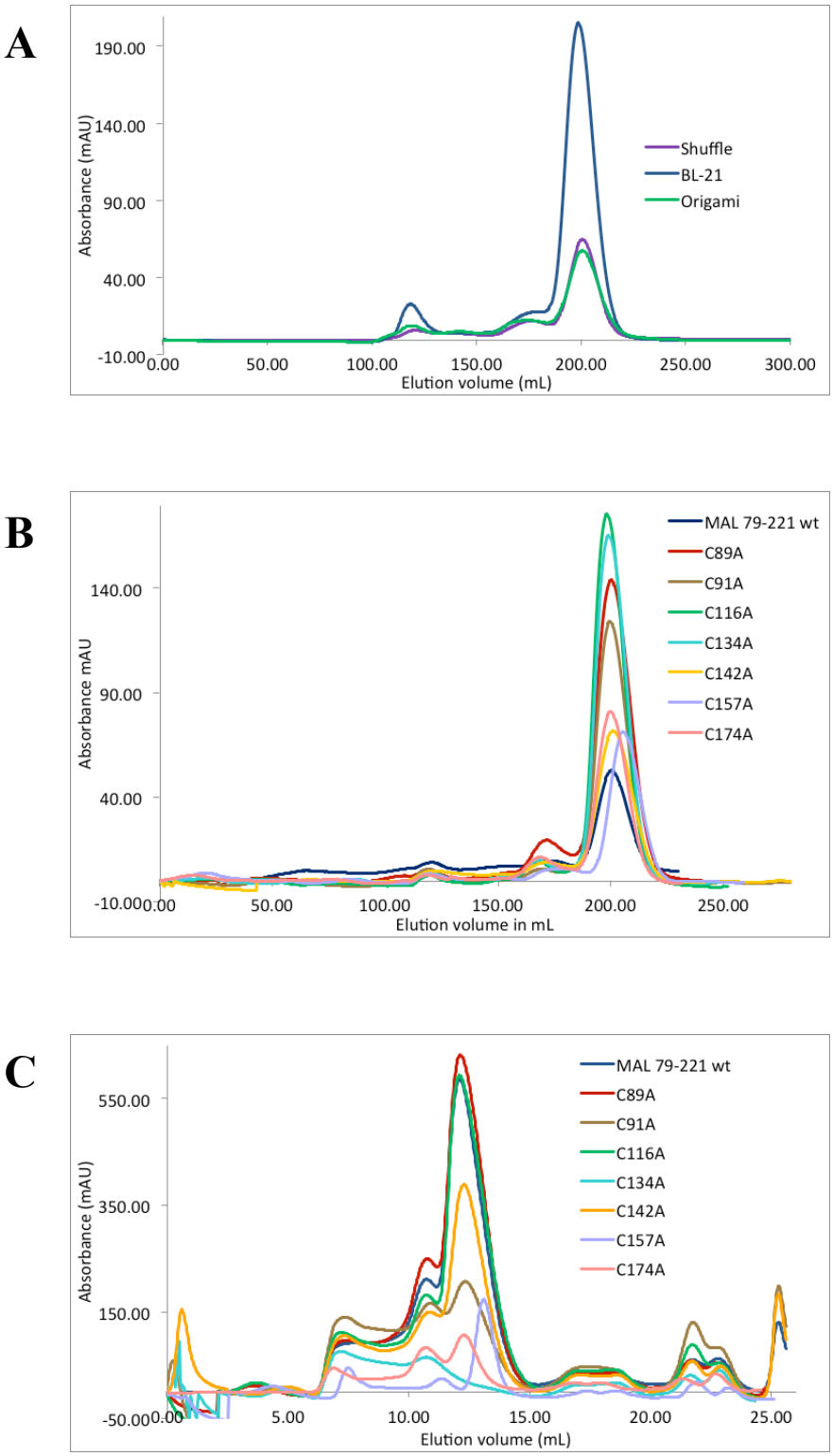
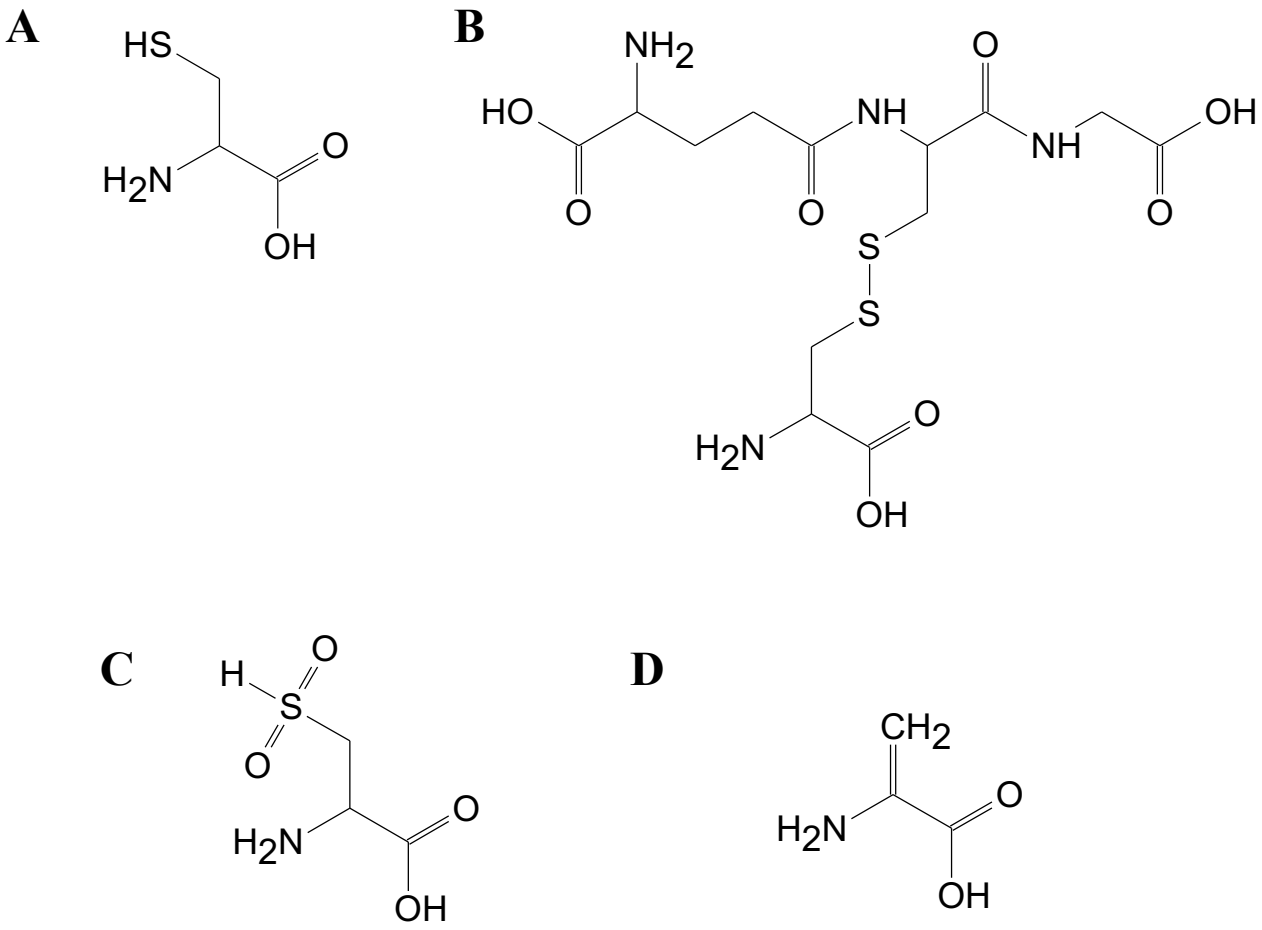
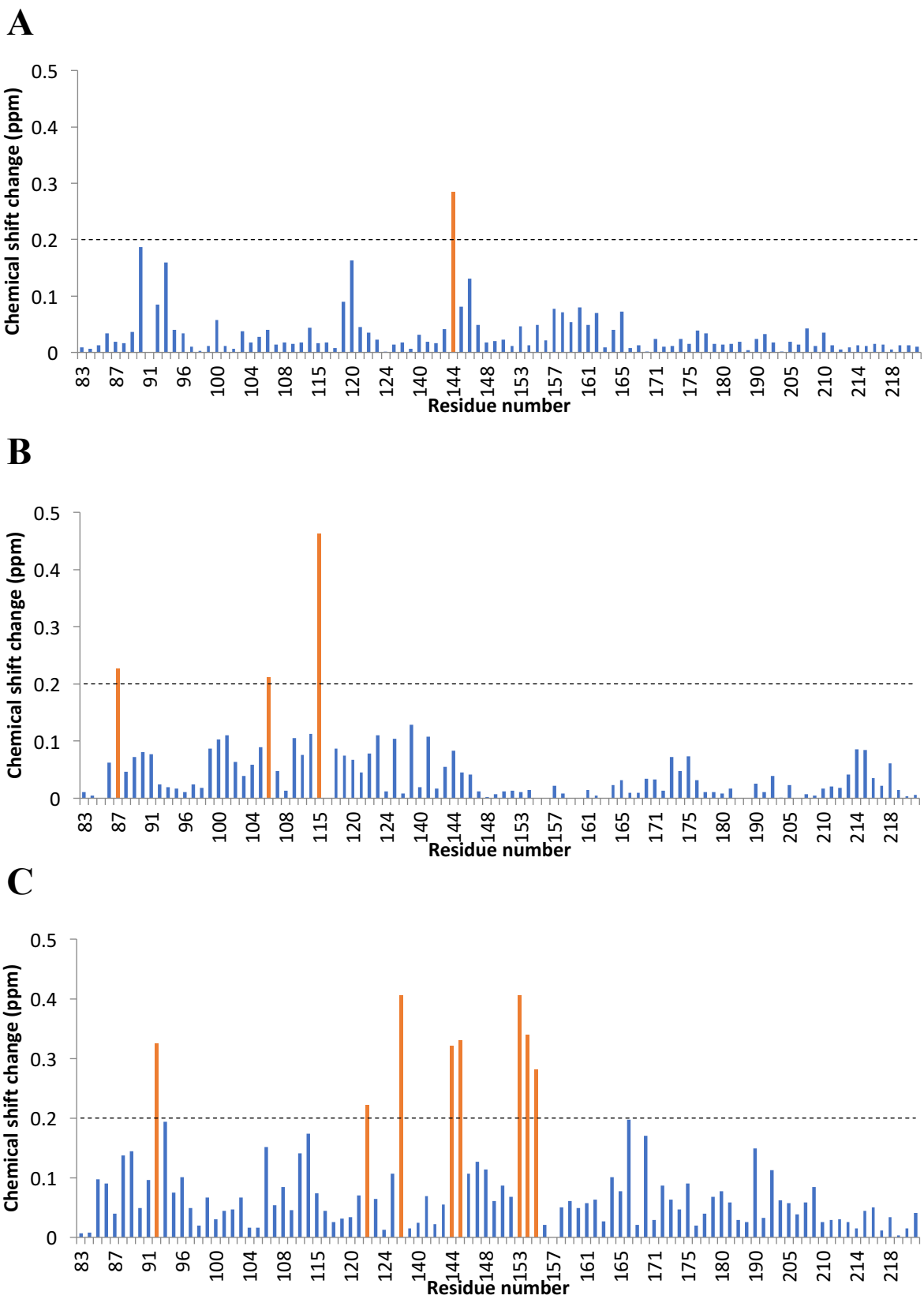


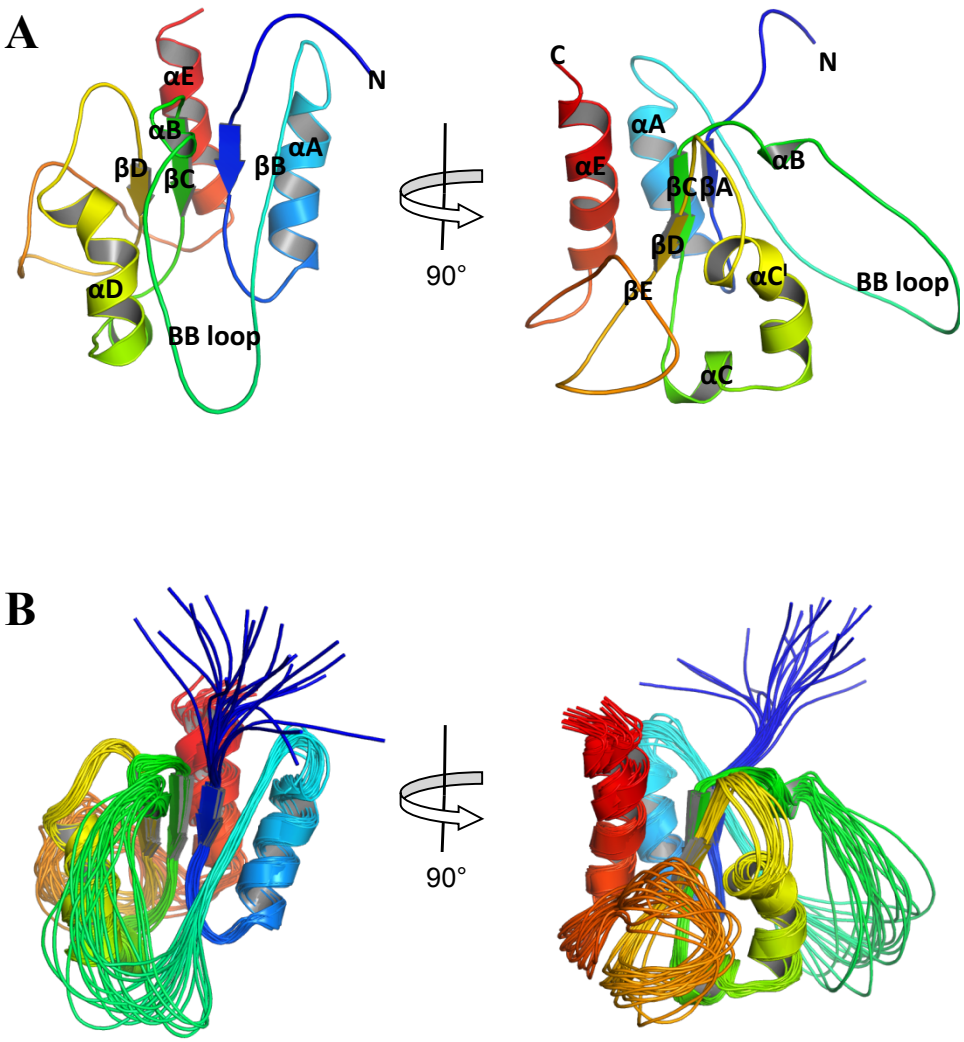
SI Appendix, Fig. S1. Size-exclusion chromatography of MAL^{TIR} and its cysteine mutants in different *E. coli* strains. (A) Size-exclusion chromatography of MAL^{TIR} produced in three different *E. coli* cell types. (B) Size-exclusion chromatography of MAL^{TIR} and single-cysteine mutants produced in BL21 *E. coli* cells. (C) Size-exclusion chromatography of MAL^{TIR} and single-cysteine mutants produced in SHuffle *E. coli* cells. Each protein was eluted from a Superdex 75 26/600 (A, B) or Superdex 75 10/300 (C) (GE Healthcare) columns in a buffer consisting of 20 mM Tris (pH 8.6) with 200 mM NaCl.



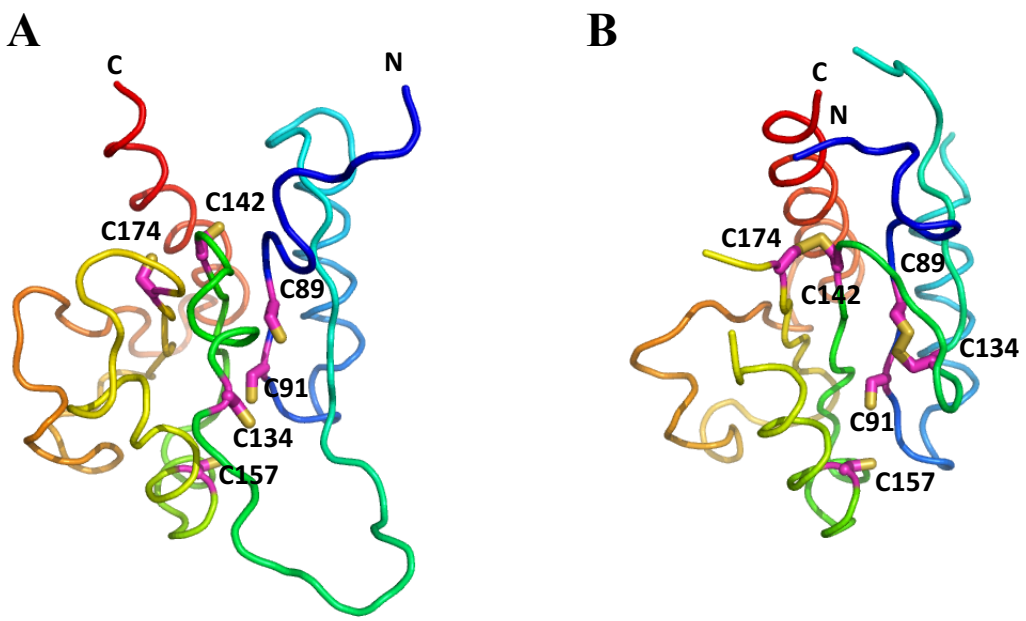
SI Appendix, Fig. S2. Structure of the amino-acid L-cysteine (A), and its modifications *S*-glutathionylation (B), dioxidation (C) and dehydroalanine (D).



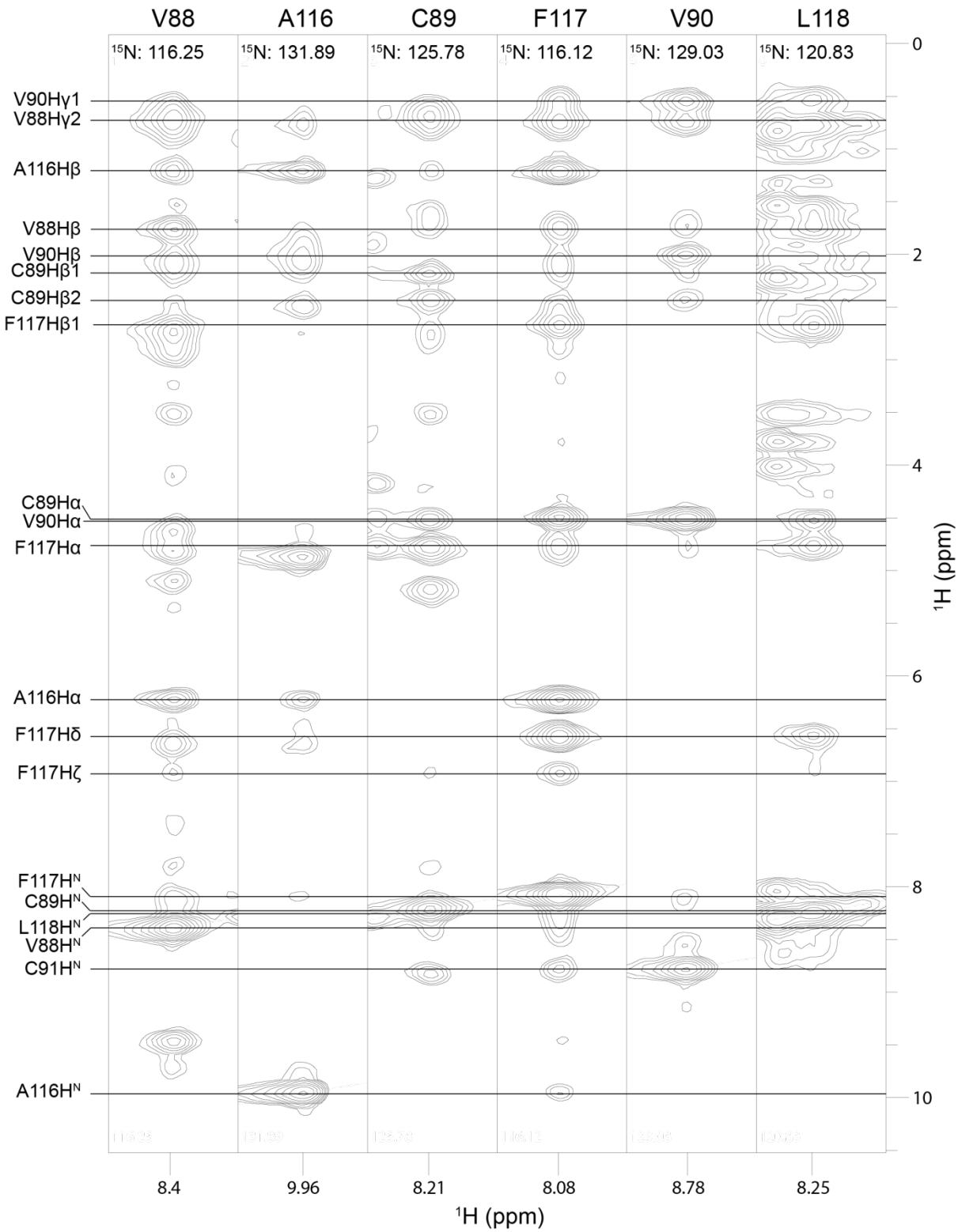
SI Appendix, Fig. S3. Comparison of chemical shifts between the (A) C91A, (B) C116A and (C) C157A mutants and the wild-type MAL^{TIR} protein. The following residues were not assigned in at least one of the spectra and were therefore excluded from the analysis: 94, 110-112, 119, 125-128, 131-136, 128,139, 149, 155, 169-170, 177, 181, 183-185, 188-189, 192-198, 201-203, 207.



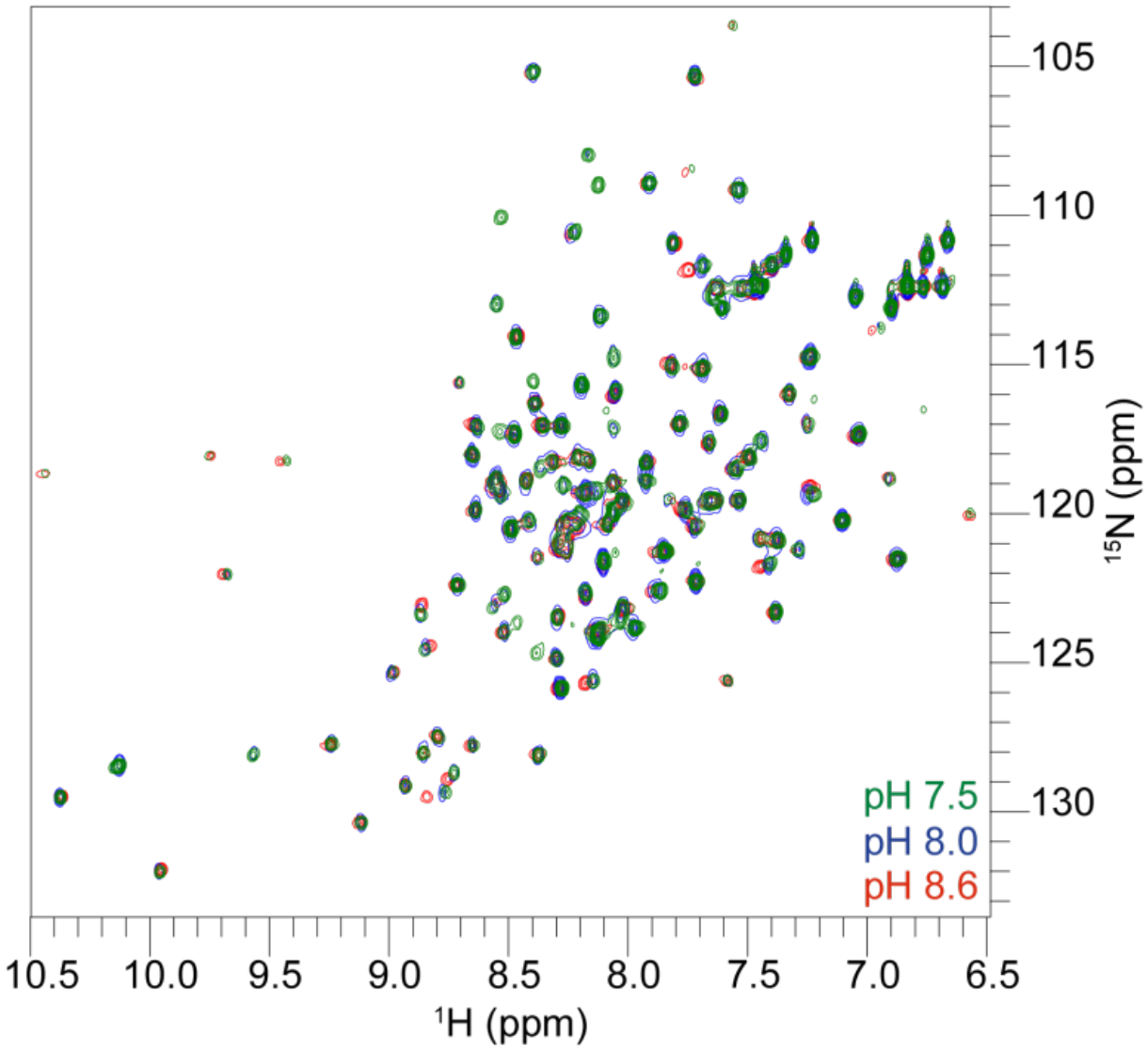
SI Appendix, Fig. S4. (A) NMR solution structure of human MAL^{TIRC116A}. The views are related by a rotation of 90° around the y-axis. (B) Top-twenty lowest-energy models, showing the inherent flexibility of the BB-loop in green. The views are related by a rotation of 90° around the y-axis.



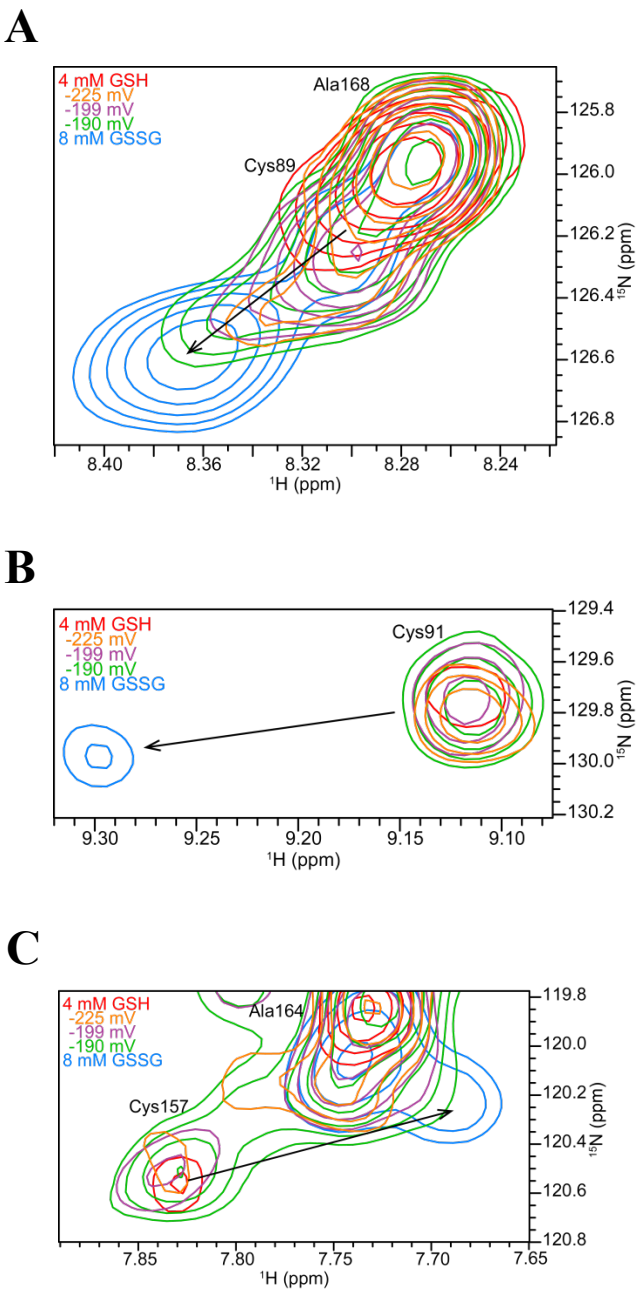
SI Appendix, Fig. S5. (A) NMR solution structure of human $MAL^{TIRC116A}$ (in ribbon representation, colored continuously from N [blue] to C-terminus [red]), highlighting the side-chains of the cysteine residues (in stick representation, with sulfurs in yellow and carbons in magenta). **(**B) Crystal structure of human MAL^{TIR}, shown as in A.



SI Appendix, Fig. S6. ^{15}N-NOESY strips from MAL^{TIRC116A} exhibiting short to medium-ranged NOE cross-peaks between residues situated in the βA strand (V88, C89, V90) and the βB strand (A116, F117, L118). The large number of atoms detected between the residues indicates that these residues are in close proximity to one another.



SI Appendix, Fig. S7. Overlay of ^{1}H-^{15}N-HSQC spectra of $MAL^{TIRC116A}$ at pH 7.5 (green), 8.0 (blue) and 8.6 (red).



SI Appendix, Fig. S8. Cysteine residues C89 (A), C91 (B) and C157 (C) show ^{1}H and ^{15}N shifts following exposure to increasingly oxidising conditions.

Movie Still S1. Still image from Movie S1 illustrating the transition between the crystal structure of MAL^{TIR} and the solution structure of $MAL^{TIRC116A}$ (left to right). The structures are shown in cartoon representation, with the side-chains of C89, C134 and P125 highlighted in stick representation. The movie was generated using UCSF Chimera [1].

Table S1. Redox state of cysteine residues in MAL^{TIR} produced in different cell types or exposed to redox reagents, as determined by the Ellman's assay.

Wild-type MAL^{TIR} growth condition	**Reduced**	**Oxidized**	**% Reduced**
BL-21 purified with 10 mM DTT	7	0	100
BL-21	6	1	90
SHuffle	4	3	57
Origami	4	3	53
H_2O_2 (0.03 mM)	0	7	0

Table S2. Number of reduced and oxidised cysteine residues in MAL^{TIR} and its mutants produced in SHuffle *E. coli* cells.

MAL mutant	**Oxidised cysteines**	**Reduced cysteines**
wild-type	3	4
C89A	3	3
C91A	5	1
C116A	3	3
C134A	-	-
C142A	2	4
C157A	3-4	2-3
C174A	3	3

Table S3. Modifications detected using mass spectrometry, following the addition of oxidized glutathione.

Cysteine residue	**Tris pH 8.6**	**Hepes pH 7.5**
89	Dehydroalanine	Dioxidation
91	Glutathionylation, dioxidation	Glutathionylation, dehydroalanine
116	Glutathionylation	Glutathionylation
134	Dehydroalanine	Glutathionylation, dehydroalanine
142	Glutathionylation, dioxidation	Glutathionylation, dioxidation
157	Glutathionylation	Glutathionylation
174	Glutathionylation	Glutathionylation

Table S4. RMSD values of ^{1}H and ^{15}N chemical shifts for cysteine-to-alanine mutants of MAL^{TIR} compared to the wild-type protein.

Cysteine mutant	**RMSD (ppm)**
C89A	0.036
C91A	0.054
C116A	0.075
C134A	0.028
C142A	0.047
C157A	0.120
C174A	0.062

Table S5. Pairwise changes of ^{1}H and ^{15}N chemical shifts between wild-type MAL^{TIR} and the cysteine-to-alanine mutants.

Residue number	C89A/WT	C91A/WT	C116A/WT	C134A/WT	C142A/WT	C157A/WT	C174A/WT
83	0.00624	0.01300	0.01400	0.01200	0.01300	0.00956	0.02200
84	0.02100	0.00840	0.00536	0.00901	0.00273	0.00833	0.04900
85	0.15200	0.01700	0.00000	0.00000	0.06800	0.12700	0.23600
86	0.08100	0.03900	0.07400	0.09200	0.10600	0.10100	0.02600
87	0.06600	0.02300	0.27400	0.00930	0.01500	0.05600	0.02100
88	0.01200	0.02100	0.05000	0.01700	0.15000	0.19000	0.04200
89		0.05000	0.09700	0.03400	0.01100	0.20400	0.14100
90	0.17900	0.23000	0.10200	0.08500	0.02300	0.05100	0.03400
91	0.03700	0.27200	0.10600	0.01100	0.01900	0.11400	0.01200
92	0.07100	0.10800	0.03600	0.00362	0.04500	0.33600	0.04700
93	0.02900	0.19500	0.24000	0.00665	0.01000	0.25800	0.04700
94							
95	0.00424	0.04700	0.02300	0.01300	0.01400	0.10600	0.00961
96	0.03100	0.04800	0.01100	0.02700	0.01800	0.12700	0.01500
97	0.01100	0.01300	0.03500	0.00840	0.01800	0.06800	0.01700
98	0.01600	0.00324	0.02600	0.01200	0.01400	0.02300	0.01400
99	0.02500	0.01400	0.09300	0.02100	0.01700	0.07700	0.01100
100	0.01900	0.07000	0.12700	0.03100	0.01200	0.03000	0.00883
101	0.02200	0.01500	0.14700	0.00730	0.03600	0.06300	0.01900
102	0.01300	0.00713	0.08900	0.00956	0.09100	0.06200	0.01400
103	0.00765	0.04100	0.05000	0.01400	0.03000	0.08500	0.01800
104	0.03800	0.02500	0.08300	0.08400	0.11400	0.02200	0.03000
105	0.01100	0.03900	0.12400	0.01300	0.01600	0.02300	0.01700
106	0.00306	0.05300	0.27900	0.08100	0.00435	0.16100	0.01600
107	0.03800	0.01600	0.04900	0.01900	0.03700	0.07500	0.03700
108	0.01400	0.02100	0.01800	0.02200	0.02000	0.11700	0.00932
109	0.01600	0.02100	0.14400	0.02200	0.02500	0.04800	0.00857
110							
111							

112							
113	0.02000	0.02000	0.08700	0.01800	0.00966	0.03600	0.02500
114	0.05100	0.05700	0.13600	0.02800	0.09400	0.19900	0.13400
115	0.01200	0.02100	0.65400	0.01300	0.03300	0.10400	0.04700
116							
117	0.11000	0.10000	0.11300	0.01700	0.01700	0.02800	0.27000
118	0.08200	0.11100	0.08200	0.00947	0.12900	0.03900	0.11300
119							
120	0.16400	0.22400	0.09100	0.12300	0.15700	0.04700	0.04600
121	0.11800	0.04500	0.05000	0.10900	0.01800	0.09800	0.06200
122	0.05600	0.04400	0.09600	0.07200	0.14000	0.25200	0.05800
123	0.02000	0.02700	0.14600	0.01700	0.01100	0.08400	0.07000
124	0.01000	0.00126	0.01600	0.00870	0.05500	0.01700	0.00500
125							
126							
127							
128							
129	0.01800	0.01900	0.11000	0.03300	0.05500	0.14400	0.02100
130	0.03800	0.01700	0.01100	0.05400	0.01400	0.08900	0.06800
131							
132							
133							
134							
135							
136							
137	0.00984	0.00781	0.14200	0.01100	0.03600	0.02200	0.02800
138							
139							
140	0.03600	0.03700	0.02700	0.03600	0.03200	0.02700	0.03500
141	0.10900	0.02100	0.12300	0.06800	0.21100	0.08200	0.00650

142	0.05100	0.02300	0.02000	0.05000	0.31000	0.03000	0.12200
143	0.09600	0.05600	0.06300	0.06000	0.18400	0.07300	0.13300
144	0.07700	0.33900	0.11400	0.08600	0.04000	0.43300	0.19200
145	0.03300	0.11200	0.04700	0.02600	0.11100	0.18400	0.09300
146	0.02400	0.16100	0.05400	0.01100	0.01400	0.15000	0.01500
147	0.00172	0.06900	0.01600	0.01500	0.02300	0.17700	0.00284
148	0.01800	0.02500	0.00238	0.00982	0.02200	0.16100	0.00000
149							
150	0.02400	0.02500	0.00860	0.02000	0.02200	0.07400	0.01000
151	0.01300	0.03000	0.01600	0.01200	0.02600	0.12300	0.00000
152	0.01600	0.01600	0.02000	0.00872	0.01900	0.09300	0.00196
153	0.00875	0.06500	0.01500	0.02200	0.01400	0.41600	0.00747
154	0.02600	0.01400	0.01300	0.00311	0.01600	0.36200	0.00115
155							
156	0.01400	0.23000	0.00000	0.00000	0.01900	0.02100	0.01400
157	0.02500	0.08900	0.02500	0.00980	0.01400		0.01200
158	0.02500	0.08300	0.01100	0.02400	0.04600	0.06500	0.01400
159	0.09300	0.07100	0.00000	0.00000	0.13200	0.08300	0.13800
160	0.03200	0.11300	0.00000	0.00717	0.01500	0.06900	0.04900
161	0.02000	0.06500	0.01700	0.03700	0.02800	0.08000	0.00307
162	0.00931	0.07400	0.00587	0.02200	0.03100	0.08200	0.04400
163	0.01000	0.00930	0.00000	0.02400	0.01000	0.03200	0.06500
164	0.02000	0.04500	0.03000	0.04000	0.03500	0.11100	0.11800
165	0.02400	0.09600	0.04400	0.02100	0.03000	0.10900	0.13700
166	0.02900	0.11000	0.00972	0.03300	0.00992	0.26100	0.07500
167	0.03400	0.01800	0.01400	0.05100	0.04100	0.02800	0.05100
168	0.02300	0.00222	0.04100	0.04400	0.07500	0.21200	0.09000
169							
170							
171	0.02900	0.03300	0.04000	0.05300	0.03300	0.04100	0.08900

172	0.01100	0.01000	0.01300	0.05300	0.05700	0.09900	0.02500
173	0.02400	0.01600	0.08200	0.00484	0.10900	0.08800	0.53300
174	0.03500	0.02500	0.06700	0.01600	0.11500	0.04900	
175	0.05400	0.02300	0.10300	0.01700	0.05700	0.12700	0.09600
176	0.04900	0.05200	0.03300	0.01100	0.02500	0.02500	0.09900
177							
178	0.01700	0.03800	0.01200	0.00233	0.05700	0.05400	0.02300
179	0.01700	0.02000	0.01200	0.01100	0.00732	0.09600	0.04500
180	0.01400	0.01900	0.01100	0.02000	0.06253	0.09600	0.01100
181							
182	0.01800	0.02200	0.02300	0.01500	0.01400	0.07700	0.01900
183							
184							
185							
186	0.01300	0.02100	0.00000	0.00000	0.01200	0.04100	0.01800
187	0.00000	0.00504	0.00000	0.00000	0.00757	0.03500	0.10500
188							
189							
190	0.01100	0.03000	0.02500	0.00950	0.00317	0.20000	0.04800
191	0.01200	0.04600	0.01500	0.01600	0.01600	0.03700	0.00169
192							
193							
194							
195							
196							
197							
198							
199	0.00392	0.02400	0.04600	0.01200	0.01100	0.15300	0.02400
200	0.00844	0.00202	0.00250	0.01100	0.02400	0.07700	0.01400
201							

202							
203							
204	0.00972	0.00824	0.02300	0.01300	0.01500	0.01000	0.00807
205	0.01300	0.02700	0.03000	0.01100	0.02200	0.08000	0.02000
206	0.00000	0.02000	0.00000	0.00000	0.00779	0.05500	0.01400
207							
208	0.05100	0.04700	0.01000	0.05000	0.00821	0.07500	0.03400
209	0.01100	0.01600	0.00689	0.01200	0.05500	0.11500	0.08400
210	0.01600	0.04600	0.01900	0.01900	0.04700	0.03500	0.02400
211	0.02100	0.01700	0.03000	0.01500	0.02200	0.04200	0.00775
212	0.00677	0.00652	0.02200	0.01100	0.05000	0.03100	0.05500
213	0.03000	0.01300	0.05200	0.01100	0.01200	0.03300	0.12300
214	0.04900	0.01700	0.11600	0.04100	0.03600	0.01800	0.00652
215	0.01300	0.01600	0.09700	0.01300	0.02100	0.05100	0.01800
216	0.00443	0.02000	0.05000	0.01400	0.03300	0.06400	0.05500
217	0.01500	0.01900	0.02900	0.01000	0.03000	0.01500	0.02500
218	0.03800	0.00506	0.06500	0.00923	0.06500	0.04300	0.02800
219	0.00783	0.01600	0.02100	0.01300	0.02300	0.00563	0.00707
220	0.01300	0.01700	0.00323	0.02000	0.01500	0.02200	0.01000
221	0.01300	0.01400	0.00776	0.00930	0.02100	0.04800	0.01600

Table S6. NMR structure statistics[a] for MAL^{TIRC116A}

Experimental restraints[b]		
	Inter-proton distance restraints	
	Intra-residue	362
	Sequential	422
	Medium-range (i–j < 5)	317
	Long-range (i–j > 5)	262
	Dihedral-angle restraints	216
	Total number of restraints per residue	11.12
Mean RMSD of the 20-structure ensemble (Å)[c]		
	Backbone atoms (residues 85–120, 134–179 & 204–220)	0.94 ± 0.17
	All heavy atoms (residues 85–120, 134–179 & 204–220)	1.28 ± 0.14
Stereochemical quality[d]		
	Residues in most favoured Ramachandran region (%)	78.0
	Ramachandran outliers (%)	0 ± 0

[a]All statistics are given as mean ± SD.
[b]Only structurally relevant restraints, as defined by CYANA, are included.
[c]Mean RMSD calculated over the entire ensemble of 20 structures.
[d]As reported by CYANA [2].

Table S7. RMSD (ppm) comparison between the ^{1}H and ^{15}N chemical shifts of MAL^{TIR} nuclei at pH 8.0 and 8.6 compared to 7.5.

pH	**7.5**
8.0	0.006
8.6	0.017

SI Appendix References:

1. Pettersen, E.F., et al., *UCSF Chimera--a visualization system for exploratory research and analysis.* J Comput Chem, 2004. **25**(13): p. 1605-12.

2. Vranken, W.F., et al., *The CCPN data model for NMR spectroscopy: development of a software pipeline.* Proteins, 2005. **59**(4): p. 687-96.